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The response of soil microbial communities to the infection of kauri (*Agathis australis*) seedlings with *Phytophthora agathidicida*

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Abstract

New Zealand's ancient kauri (*Agathis australis*) forests are under threat from the spread of dieback disease, caused by the soil-borne pathogen *Phytophthora agathidicida*. Characterizing the response of the soil microbiota to the infection of kauri with *P. agathidicida* is essential to identify how they may form a protective response to pathogen invasion and disease expression. This study infected 18-month-old kauri seedlings with a standardized inoculum load of *P. agathidicida* for 6 weeks under controlled environmental conditions. Following this, changes in the diversity, composition and biomass of soil microbial communities associated with kauri seedlings were characterized using high-throughput 16S rRNA and ITS gene region sequencing and phospholipid fatty acid analysis. Significant differences were found in the composition of soil bacterial communities associated with inoculated and non-inoculated kauri seedlings. Furthermore, soils of inoculated seedlings had a significantly higher relative abundance of bacteria previously reported to be associated with plant disease suppression, which included several members of the Firmicutes. Significant reductions were found in the fungal: bacterial biomass of soils from inoculated seedlings. This finding contrasts to previous field-based research that observed an increased diversity of soil fungal communities associated with diseased kauri in old growth kauri forests. Further research that aims to isolate members of the kauri soil microbiota and study their interactions with *P. agathidicida* is required for us to begin selecting potential biocontrol agents against kauri dieback.

KEYWORDS
Agathis australis, disease suppressive soils, kauri dieback, *Phytophthora agathidicida*, seedling bioassay, soil microbiota

1 | INTRODUCTION

New Zealand's ancient kauri (*Agathis australis*) forests are currently threatened by the spread of kauri dieback, a root and collar rot disease caused by *Phytophthora agathidicida* (Beever et al., 2009; Weir et al., 2015). *P. agathidicida* is a highly virulent, soil-borne pathogen that is spread across kauri forests through the movement of contaminated soil, soil water and root pieces (Beever et al., 2009; Bellgard et al., 2013). Kauri dieback is now widely distributed across most of the remaining natural geographic range of kauri (Bradshaw et al.,

2020; Weir et al., 2015), and urgent action is needed to manage the spread of the disease.

The soil microbiota have numerous functional roles in supporting plant health and providing defence against soil-borne pathogens (Berendsen et al., 2012). The interactions between invading pathogens and the resident soil microbiota can determine the outcome of disease progression, with resident microorganisms that establish antagonistic interactions being those which can successfully suppress pathogen development (Raaijmakers et al., 2009). Studying the microbial communities associated with disease outbreaks can identify soil microorganisms that are responding to plant attack following pathogen invasion (Galiana et al., 2011). This study characterized the changes in diversity, composition and biomass of soil microbial communities associated with kauri seedlings 6 weeks following their inoculation with *P. agathidicida*. By doing so, we aimed to assess how the kauri soil microbiota responds to the initial stages of pathogen invasion and seedling disease expression.

Previous research by Byers et al. (2020) identified significant differences in the diversity and composition of soil microbial communities associated with asymptomatic and symptomatic mature kauri (approximately 200 years old) across Waipoua Forest (Northland Region, New Zealand). In addition, microbial taxa with previously reported roles in providing soil disease suppression such as *Penicillium*, *Trichoderma* and *Pseudomonas* (Garbeva et al., 2004) had a significantly higher relative abundance in asymptomatic kauri soils compared to symptomatic kauri soils. This study aimed to build on the findings of Byers, Condron, O'Callaghan, et al. (2020) by standardizing several variables which were not possible to standardize in the field, such as kauri tree age, the time since initial infection and the inoculum load of *P. agathidicida*. Due to the threatened conservation status and high cultural value of kauri, infecting mature kauri using controlled field experiments was not possible for this study (De Lange et al., 2013; Lambert et al., 2018). Therefore, this study infected young kauri seedlings with a standardized load of *P. agathidicida* to examine the response of the soil microbiota to infection under controlled experimental conditions. The soils of kauri seedlings were sampled 6 weeks following inoculation, and this short-term 6-week inoculation period was selected to identify microbial taxa which were primary respondents to seedling infection. The diversity and composition of soil microbial communities associated with inoculated and non-inoculated kauri seedlings were characterized using high-throughput 16S rRNA and ITS gene region sequencing. In addition, phospholipid fatty acid analysis (PLFA) was performed to quantify the differences in viable microbial biomass between soils from inoculated and non-inoculated seedlings (Nkongolo & Narendrula-Kotha, 2020).

2 | METHODS

2.1 | Soil sampling

To obtain kauri forest soils suitable for use as potting mix in the seedling bioassays, organic layer soil samples were collected from 18 asymptomatic kauri trees across Waipoua Forest (Northland Region, New Zealand) according to the sampling methods as outlined by Byers, Condron, O'Callaghan, et al. (2020). The GPS coordinates of the kauri trees sampled across Waipoua Forest are shown in Table S1, Supplementary Materials. Following soil sampling, the presence or absence of *P. agathidicida* in kauri soils was determined using a soil baiting bioassay (Bellgard et al., 2013) and TaqMan real-time PCR (McDougal et al., 2014; Than et al., 2013). Soil samples which tested negative for *P. agathidicida* across both pathogen detection methods were used as potting mix. The results of the pathogen bioassays are shown in Table S1, Supplementary Materials. The sampling locations of the soil samples which qualified for use as seedling potting mix are shown in Figure S1, Supplementary Materials.

2.2 | Seedling bioassays

For the seedling bioassays, 60 18-month-old kauri seedlings of approximately 20 cm in height were sourced from the Auckland Botanical Gardens (Auckland, New Zealand). Prior to transplant, seedlings had been grown in a C.A.N fines screened (65%) and washed sand potting mix (35%). Kauri seedlings were transplanted into 1 litre plant pots that were filled with 500 g of uninfected kauri forest soil, with all excess potting material being removed from the roots while taking care to avoid root damage. Following transplant, seedlings were left to equilibrate in their new soils for 2 weeks and monitored for symptoms of ill-health or transplant damage. Seven seedlings showed signs of transplant damage and were discarded, which left 53 seedlings for the experiment.

The *P. agathidicida* strain NZFS3770 was used as a pathogen inoculum source for the seedling bioassay. Cultures were maintained at 22°C in darkness on 20% clarified V8 agar (Lawrence et al., 2017). Prior to setting up the seedling bioassay, lupin (*Lupinus angustifolius*) seedlings were infected to confirm pathogenicity of *P. agathidicida* NZFS3770 (Lewis, 2018). To prepare the inoculum source, 5 mm^2 agar plugs of *P. agathidicida* were incubated in 10 ml of 2% concentrate V8 broth in darkness at 22°C for 24–36 h, or until sufficient mycelial growth of 3 cm was observed (Horner & Hough, 2014; Lawrence et al., 2017). An image of a mycelial mat of *P. agathidicida* is shown in Figure S2, Supplementary Materials. These mats of *P. agathidicida* mycelium were used to infect 27 kauri seedlings. To ensure non-inoculated seedlings were also loaded with nutrient-rich V8 agar and broth, 26 kauri seedlings were inoculated with blank V8 agar plugs which had been incubated in V8 broth in darkness at 22°C for 24–36 h as a control. Each kauri seedling was inoculated with five mycelial mats, or blank V8 agar plugs for the control seedlings, which were spaced evenly around the seedling. Mycelial mats were inserted 10 cm deep into the soil profile to be within the seedling root zone (Davison & Tay, 1987). To ensure sustained infection during the experiment, seedlings were re-inoculated on week two and week three with three *P. agathidicida* mycelial mats, while control (non-inoculated) seedlings were re-inoculated with three blank V8 agar plugs. Following inoculation, inoculated and non-inoculated seedlings were held at 100% water holding capacity for 1 week

promote zoospore motility of *P. agathidicida* and stimulate seedling infection (Davison & Tay, 1987). Saucers containing the seedling pots were monitored and, if necessary, drained to prevent a build-up of water. For the remainder of the experiment, seedlings were maintained at 50% of soil water holding capacity. For the duration of the experiment, seedlings were incubated in growth chambers (Sanyo Versatile Environmental Test Chamber MLR-351) that were maintained at a constant temperature of 18°C (Horner & Hough, 2014) with cycling photoperiods of 12 h light and 12 h darkness.

Six weeks following their inoculation *P. agathidicida*, seedlings were destructively sampled and their soils were collected for analysis. Harvested seedlings were measured for seedling length (mm), shoot weight (g), root weight (g) and root: shoot ratio. At the end of the experiment, differences in these measures of seedling biomass between inoculated and non-inoculated seedlings were tested for significance using Student's *t* tests. For each seedling, the soil within a 2 cm^2 area surrounding their root system (approximately 10 cm depth of the soil profile) was collected and sieved to remove plant material and homogenise sample. For soil RNA extractions, 10 g subsamples were preserved in LifeGuard Soil Preservation Solution (Qiagen) and stored at −80°C. For soil DNA extractions, 10 g subsamples were stored at −20°C. For PLFA analysis, 10 to 20 g subsamples were stored at 4°C.

2.3 | Soil DNA extractions and high-throughput sequencing

Soil DNA extractions and high-throughput sequencing followed methods outlined by Byers, Condron, Donavan, et al. (2020) and subsequent bioinformatics analysis followed methods outlined by Byers, Condron, O'Callaghan, et al. (2020). Briefly, DNA extractions were performed on three 0.25 g soil samples collected from each seedling. Following quality control checks, DNA extracts were sent to Novogene Co., Ltd for library preparation and sequencing of the bacterial 16S rRNA (V3–V4) and the fungal ITS (ITS2) gene region on an Illumina HiSeq platform. Initial processing and quality filtering of sequencing reads were performed using QIIME V 1.7.0 (Caporaso et al., 2010). Sequencing reads were clustered into OTUs at 99% sequence similarity using QIIME 2 2019.4 (Bolyen et al., 2019), and a representative sequence from each OTU was identified taxonomically using the reference databases 'Green Genes 13.8' for 16S rRNA classification and 'UNITE 18.11.2018' for ITS classification. OTU frequency tables were filtered to remove non-bacterial or fungal taxa and low abundance taxa of <10 read counts. To even differences in sampling depth between samples, OTU frequency tables were rarefied to minimum sampling depth (4000 counts per sample for fungi and 17,000 counts per sample for bacteria).

2.4 | Statistical analyses

Alpha diversity analyses were performed on rarefied OTU tables using Qiime 2 'diversity' plugin, and differences in the alpha diversity values between inoculated and non-inoculated seedlings were tested for significance using Kruskal–Wallis chi-squared tests. To assess differences in beta diversity between inoculated and non-inoculated seedlings, Non-metric Multidimensional Scaling (NMDS) was performed on Bray–Curtis dissimilarity matrices calculated on rarefied fungal and bacterial OTU tables (Oksanen et al., 2019; McMurdie & Holmes, 2013). Differences in dissimilarity scores between inoculated and non-inoculated seedlings were tested for significance using analysis of similarities (ANOSIM). Kruskal–Wallis chi-squared tests were used to identify taxonomic ranks which had significantly different relative abundances between inoculated and non-inoculated seedlings. DESeq2 analysis was used to identify and visualize individual OTUs which had a significantly different abundance between inoculated and non-inoculated seedlings (McMurdie & Holmes, 2013; Love et al., 2014).

2.5 | Phospholipid fatty acid analysis

To prepare soils for PLFA analysis, a 5 to 10 g soil sample from 15 randomly selected inoculated seedlings and 15 randomly selected non-inoculated seedlings was freeze dried for 4 days. Freeze-dried soil samples were sent to Microbial ID, Inc. for high-throughput PLFA analysis using the Sherlock PLFA analysis system (Buyer & Sasser, 2012). Individual phospholipid fatty acids were grouped into different microbial classes based on assignments previously defined by Willers et al. (2015). Fatty acid chains 17:1 ω7c 10-methyl, 17:0 10-methyl, 18:1 ω7c 10-methyl and 18:0 10-methyl were assigned as Actinomycetes; 16:1 ω9c, 16:1 ω7c, 17:1 ω8c, 17:0 cyclo ω7c, 18:1 ω7c, 18:1 ω5c, 19:0 cyclo ω7c and 20:1 ω9c were assigned as gram-negative bacteria; 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:1 iso ω9c, 17:0 iso and 17:0 anteiso were assigned as gram-positive bacteria; 14:0, 15:0, 16:0 and 17:0 were assigned as other eubacteria; 16:1 ω5c was assigned as arbuscular mycorrhizal fungi (AM fungi); 18:1 ω9c was assigned as saprophytic fungi and 18:2 ω6c was assigned as ectomycorrhizal fungi (EM fungi). Differences in the relative abundance of each microbial group were tested for significance using Student's *t* tests.

2.6 | Quantification of *P. agathidicida* abundance

Real-time PCR assays were performed on soil cDNA extracts to confirm that inoculated seedlings tested positive for viable cells of *P. agathidicida* and that non-inoculated seedlings tested negative for *P. agathidicida* and were not contaminated during the experiment. Soil RNA extractions were performed using an RNeasy PowerSoil Total RNA kit (Qiagen) according to manufacturer's instructions. Following extraction, RNA samples were treated with DNase and converted to cDNA using an iScript gDNA Clear cDNA Synthesis Kit (Bio-Rad). To remove genomic DNA, 16 µl of DNase master mix was prepared using 0.5 µl iScript DNase, 1.5 µl iScript DNase buffer and 1 µg of RNA and nuclease-free water. Samples were incubated in a thermocycler at 25°C for 5 min then 75°C for 5 min. Following

DNase treatment, 4 µl of iScript Reverse Transcription Supermix was added to RNA samples and incubated in a thermocycler at 25°C for 5 min, 46°C for 20 min and 95°C for 1 min to obtain cDNA samples. The resulting cDNA samples were quantified for their abundance of *P. agathidicida* using a real-time PCR as described by Than et al. (2013) and McDougal et al. (2014).

3 | RESULTS

3.1 | Seedling disease expression

The results of the real-time PCR assays confirmed that all 26 inoculated seedlings tested positive for *P. agathidicida*, with the average soil cDNA abundance of *P. agathidicida* being 34.60 ± 4.93 femtograms/seedling at the end of the experiment (Table S3, Supplementary Materials). None of the non-inoculated seedlings tested positive for *P. agathidicida*. Seedling disease assessments taken at the end of the experiment found that the dry root weights, shoot weights, root: shoot ratio and seedling length were significantly lower in inoculated seedlings than non-inoculated seedlings (p-value < .05; Table S2, Supplementary Materials). All inoculated seedlings expressed symptoms of dieback including chlorosis, wilting, leaf litter loss, necrosis and mortality (Figure S3, Supplementary Materials).

3.2 | Differences in microbial diversity and composition

As shown in Table 1, there were no significant differences in the Shannon diversity or Pielou's evenness of soil fungal communities between inoculated and non-inoculated kauri seedlings. The number of observed fungal OTUs was significantly lower in inoculated seedlings compared to non-inoculated seedlings. For soil bacterial communities, the Shannon diversity, Pielou's evenness and number of observed OTUs were significantly higher in inoculated seedlings compared to non-inoculated seedlings.

Figure 1 shows the differences in fungal and bacterial community composition. There was no significant difference in the fungal community composition of soils associated with inoculated and non-inoculated seedlings (ANOSIM R = 0.050, p-value = .05). In contrast, there was a significant difference in the bacterial community composition of soils associated with inoculated and non-inoculated seedlings (ANOSIM R = 0.391, p-value < .001).

3.3 | Changes in taxonomic composition of fungal communities

The phyla Mortierellomycota (H-value = 14.23, p-value < .001) and Rozellomycota (H-value = 7.22, p-value = .007) had a significantly higher relative abundance in soils from non-inoculated seedlings compared to inoculated seedlings. Shown in Figure 2, the Botryosphaeriales (H-value = 22.23, p-value < .001) had a significantly higher relative abundance in soils from inoculated seedlings, whereas the Mortierellales (H-value = 14.23, p-value < .001) had a higher relative abundance in soils from non-inoculated seedlings.

As shown in Figure 3, 43 fungal OTUs were found in significantly higher relative abundance in soils from inoculated seedlings. These OTUs included the taxa *Hyaloscyphaceae*, *Meliniomyces*, *Pezicula*, *Helotiales*, *Auriculariales*, *Leucoagaricus*, *Byssochlamys*, *Candida*, *Herpotrichiellaceae*, *Strophariaceae*, *Tubulicium*, *Tolypocladium*, *Piskurozymaceae*, *Aspergillus* and Botryosphaeriales. Also displayed in Figure 3, 20 fungal OTUs were found to have a significantly lower relative abundance in soils from inoculated seedlings, which included the taxa *Mortierella*, *Pyronemataceae*, *Galerina*, *Absidia*, *Mucor*, *Neobulgaria*, *Hydnodontaceae*, *Pseudeurotiaceae* and *Clavariaceae*. A complete list of fungal OTUs found in significantly different relative abundance between inoculated and non-inoculated seedlings can be found in Table S4, Supplementary Materials.

TABLE 1 The mean ± standard error alpha diversity values calculated for fungal and bacterial communities in inoculated and non-inoculated seedlings

	Alpha diversity	Inoculated	Non-inoculated	Significance
Fungi	Shannon diversity	5.39 ± 0.12	5.59 ± 0.14	H-value = 0.85, p-value = .36
	Pielou's evenness	0.44 ± 0.01	0.45 ± 0.01	H-value = 0.41, p-value = .52
	Observed OTUs	4974.40 ± 108.60	5389.81 ± 133.63	H-value = 4.94, p-value = .03
Bacteria	Shannon diversity	11.93 ± 0.03	11.76 ± 0.03	H-value = 12.41, p-value < .001
	Pielou's evenness	0.85 ± 0.00	0.84 ± 0.00	H-value = 11.91, p-value < .001
	Observed OTUs	17009.19 ± 211.31	16178.69 ± 171.99	H-value = 9.69, p-value = .002

Note: The results of Kruskal–Wallis chi-squared tests are displayed which were used to test for significant differences in the alpha diversity values of inoculated and non-inoculated seedlings.

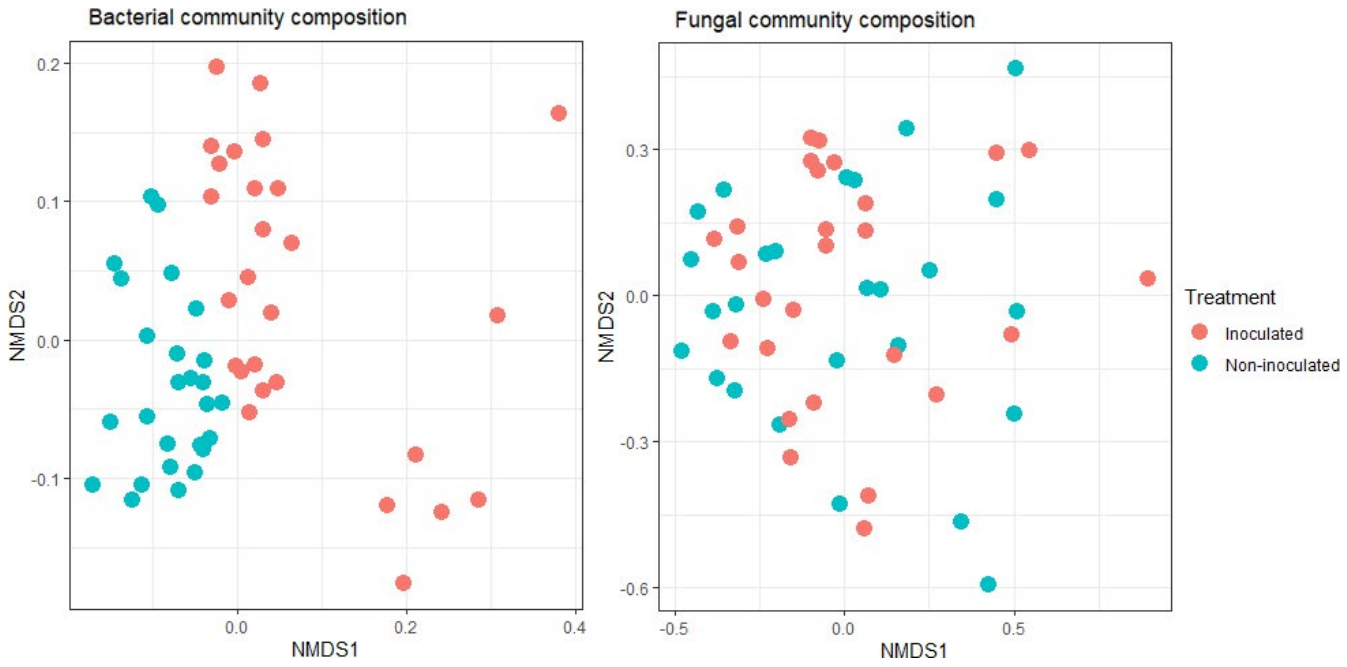

FIGURE 1 Ordination plots built using Non-metric Multidimensional Scaling (NMDS) of Bray–Curtis dissimilarity matrices which show the differences in fungal and bacterial community composition in soils associated with inoculated and non-inoculated kauri seedlings

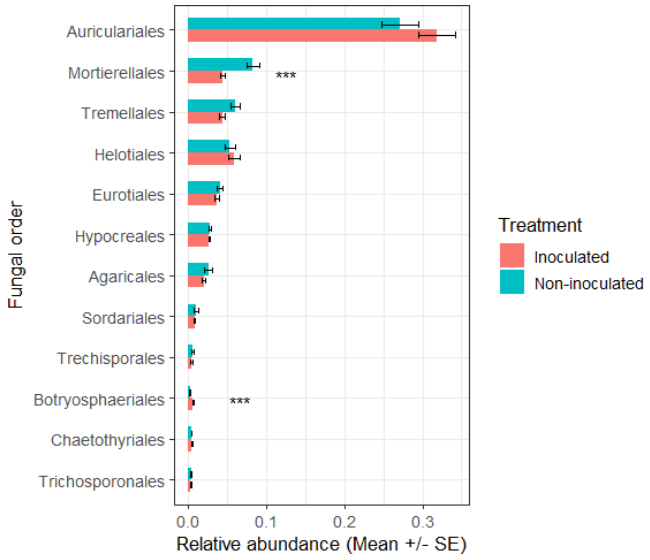

FIGURE 2 The mean ± standard error relative abundances (%) of fungal orders in soils from inoculated and non-inoculated seedlings. Significant differences in relative abundances of fungal orders were determined using Kruskal–Wallis chi-squared tests and are denoted by *, where *p*-value < .05 is *, *p*-value < .01 is **, and *p*-value < .001 is ***

3.4 | Changes in taxonomic composition of bacterial communities

The phylum Firmicutes was found in significantly higher relative abundance in soils from inoculated seedlings (*H*-value = 39, *p*-value < .001). Actinobacteria (*H*-value = 25.35, *p*-value < .001), Verrucomicrobia (*H*-value = 6.66, *p*-value = .01) and Nitrospirae (*H*-value = 7.70, *p*-value = .01) were found in significantly higher relative abundance in soils from non-inoculated seedlings. As shown in Figure 4, bacterial orders found in significantly higher relative abundance in soils from inoculated kauri seedlings were Clostridales (*H*-value = 39, *p*-value < .001), Burkholderiales (*H*-value = 33.23, *p*-value < .001) and Bacillales (*H*-value = 36.40, *p*-value < .001). Bacterial orders with a significantly higher relative abundance in soils from non-inoculated seedlings were Rhizobiales (H-value = 28.30, *p*-value < .001), Rhodospirillales (*H*-value = 18.08, *p*-value < .001), Solibacterales (*H*-value = 14.09, *p*-value < .001), Solirubrobacterales (*H*-value = 25.53, *p*-value < .001), Myxococcales (*H*-value = 7.70, *p*-value = .006), Actinomycetales (*H*-value = 17.33, *p*-value < .001), Acidimicrobiales (*H*-value = 27.92, *p*-value < .001), Gaiellales (*H*-value = 14.50, *p*-value < .001) and Chthoniobacterales (*H*-value = 8.83, *p*-value = .002).

As shown in Figure 5, 114 bacterial OTUs were found in significantly higher relative abundance in soils from inoculated seedlings. These OTUs included the taxa *Bacillus*, *Clostridium*, *Planococcus*, *Paenibacillus*, *Gallionella*, *Paludibacter*, *Geobacter*, *Herbaspirillum* and *Salinspora*. In total, 48 bacterial OTUs were found in significantly lower relative abundance in soils from inoculated seedlings, which included the taxa *Frankia*, *Actinoplanes*, *Kribbella*, *Nocardia*, *Phyllobacterium*, *Pseudonocardia*, *Pedomicrobium*, *Edaphobacter* and *Sphingomonas*. A complete list of bacterial OTUs found in significantly different relative abundance between inoculated and non-inoculated seedlings can be found in Table S5, Supplementary Materials.

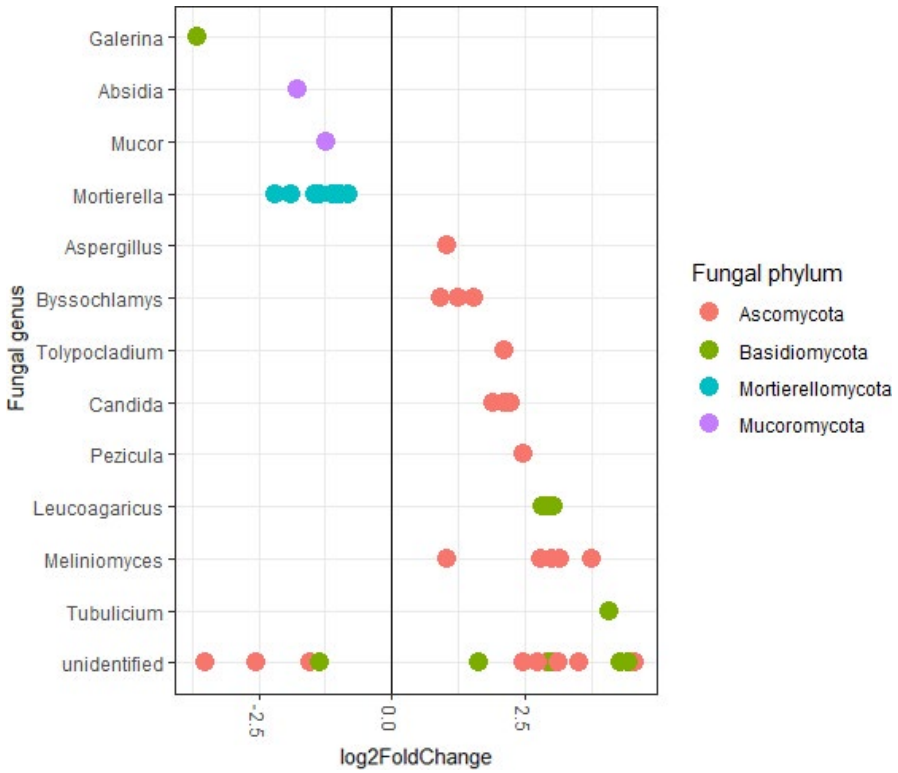

FIGURE 3 The fungal OTUs found to have a significantly different relative abundance in soils from inoculated and non-inoculated kauri seedlings. Fungal OTUs with positive 'log2FoldChange' values were found significantly higher in soils from inoculated seedlings, and fungal OTUs with negative 'log2FoldChange' values were found significantly higher in soils from non-inoculated seedlings

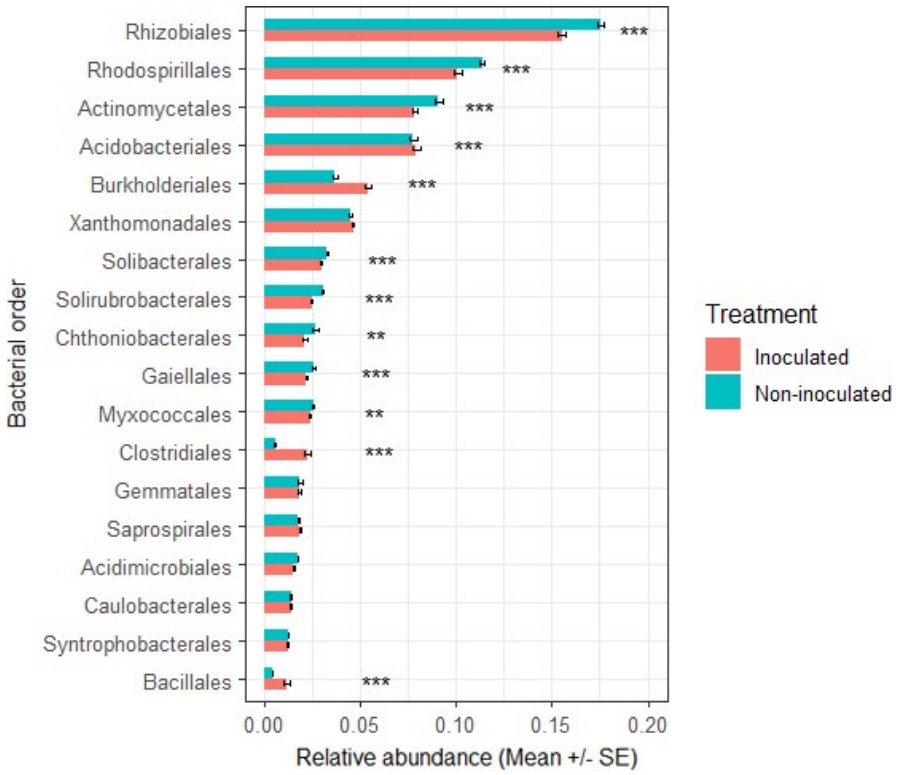

FIGURE 4 The mean ± standard error relative abundances (%) of bacterial orders in soils from inoculated and non-inoculated seedlings. Significant differences in relative abundances of bacterial orders were determined using Kruskal–Wallis chi-squared tests and are denoted by *, where p-value < .05 is *, p-value < .01 is **, and p-value < .001 is ***

3.5 | Phospholipid fatty acid analysis

As shown in Table 2, soils from inoculated seedlings had a significantly higher relative abundance of total bacteria and other eubacteria compared to soils from non-inoculated seedlings. The relative abundance of total fungi, EM fungi, Actinomycetes and the fungal: bacterial ratio was significantly lower in soils from inoculated seedlings compared to non-inoculated seedlings. There were no significant differences in the total PLFA abundance (nmol/g) between soils from inoculated and non-inoculated seedlings, nor the relative abundances of gram-positive bacteria, gram-negative bacteria, AM fungi or saprophytic fungi.

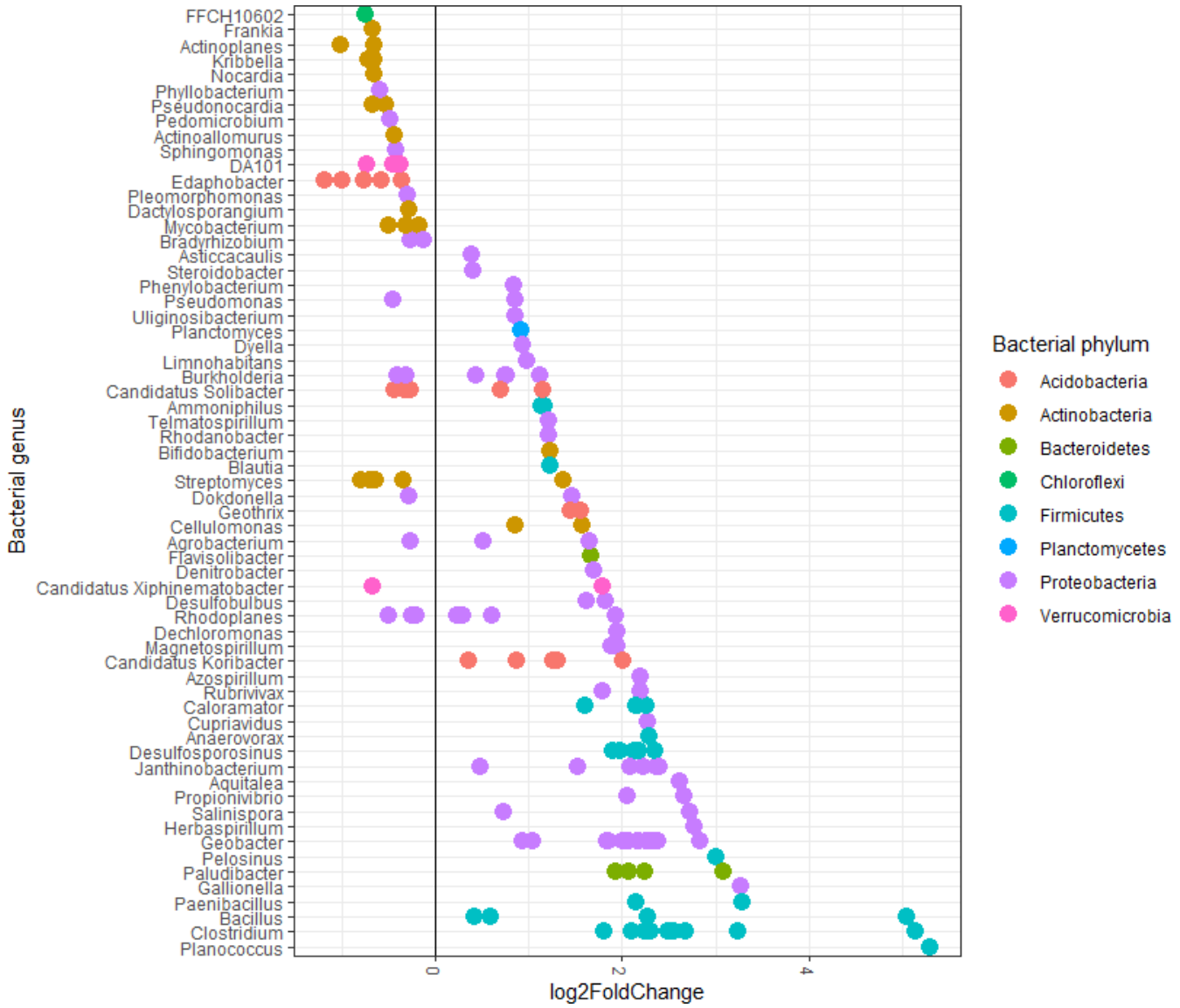

FIGURE 5 The bacterial OTUs found to have a significantly different relative abundance in soils from inoculated and non-inoculated kauri seedlings. Bacterial OTUs with positive 'log2FoldChange' values were found significantly higher in soils from inoculated seedlings, and bacterial OTUs with negative 'log2FoldChange' values were found significantly higher in soils from non-inoculated seedlings

4 | DISCUSSION

4.1 | Pronounced shifts in soil bacterial communities following seedling infection

Pronounced and significant differences were found in soil bacterial community composition between inoculated and non-inoculated seedlings, which may be explained by changes in the availability of soil nutrients and labile carbon (C) following seedling disease expression. Kauri seedlings inoculated with *P. agathidicida* experienced significant losses in their root mass, a factor which may have reduced their release of root exudates. Plant root exudates provide important inputs of easily available, carbon-rich compounds that help to sustain soil microbial activity (De Graaff et al., 2010). Inoculated seedlings may have experienced an increased abundance of bacterial taxa with oligotrophic nutritional strategies which are less dependent on a rich supply of labile C, compared to copiotrophic bacteria which prefer nutrient-rich soils with larger pools of labile C (Fierer et al., 2007). For example, the Firmicutes, including the genera *Bacillus* and *Paenibacillus*, were found in significantly higher relative abundance in soils from inoculated seedlings. The Firmicutes are commonly found abundant in low nutrient soils due to their ability to use recalcitrant carbon and inorganic nutrients (Llado et al., 2017). For example, *Bacillus* and *Paenibacillus* have demonstrated the ability to degrade phenolic compounds (Llado et al., 2017; Tian et al., 2014) of which are found in high concentrations in kauri leaf litter (Verkaik et al., 2006; Wyse et al., 2014). The increased abundance of these bacterial taxa in soils from inoculated seedlings may indicate they have a competitive advantage in the soil environment following seedling disease expression which is driving down the abundance of other bacterial taxa.

TABLE 2 The mean ± standard error relative abundance (%) of microbial groups in soils from inoculated and non-inoculated seedlings

Microbial group	Non-inoculated	Inoculated	Significance
Total PLFA (nmol/g)	481.0 ± 16.6	451.0 ± 16.5	*T*-value = 1.29, *p*-value = .21
Fungi: Bacteria ratio	0.21 ± 0.01	0.19 ± 0.00	*T*-value = 2.40, *p*-value = .02
Total bacteria (%)	81.78 ± 0.40	83.01 ± 0.29	*T*-value = 2.48, *p*-value = .02
Total fungi (%)	17.56 ± 0.40	16.39 ± 0.30	T-value = 2.35, *p*-value = .03
Gram-Negative (%)	43.08 ± 0.22	43.32 ± 0.28	*T*-value = 0.69, *p*-value = .49
Gram-Positive (%)	18.20 ± 0.37	18.32 ± 0.29	*T*-value = 0.26, *p*-value = .80
Other Eubacteria (%)	15.77 ± 0.22	17.26 ± 0.23	*T*-value = 4.73, *p*-value < .001
Saprophytic fungi (%)	10.72 ± 0.21	10.43 ± 0.16	*T*-value = 1.09, *p*-value = .28
Actinomycetes (%)	4.74 ± 0.14	2.41 ± 0.09	*T*-value = 3.65, *p*-value < .001
AM Fungi (%)	3.81 ± 0.14	3.55 ± 0.14	*T*-value = 1.33, *p*-value = .19
EM Fungi (%)	3.03 ± 0.15	2.41 ± 0.09	*T*-value = 3.49, *p*-value = .002

Note: Values for each microbial group were calculated as the sum of each group's abundance (ng/mol) relative to the total PLFA (ng/mol). The results of Student's *t* tests are displayed which were used to test for significant differences in the relative abundances of microbial groups between inoculated and non-inoculated seedlings.

The Actinomycetales had a significantly lower relative biomass and abundance in soils from inoculated seedlings. This contrasts to previous findings of Byers, Condron, O'Callaghan, et al. (2020) which observed a higher relative abundance of Actinobacteria in field soils associated with symptomatic kauri. The Actinomycetales are considered putative copiotrophs which have important roles in the degradation of soil organic matter (Ding et al., 2015; Fierer et al., 2012; Navarrete et al., 2015). The decreased abundance of the Actinomycetales in soils from inoculated seedlings may be a response to these changes in soil nutrient availability and C supply following seedling infection. In contrast, their increased abundance in symptomatic kauri field soils (Byers, Condron, O'Callaghan, et al., 2020) may have been due to the larger inputs of dead plant tissue in symptomatic field soils following tree dieback. This is supported by the findings of Yao et al. (2014) who observed a decreased biomass of the Actinomycetes in *Escherichia coli* infested soils. These declines were attributed to *E. coli* infested soils shifting from a copiotrophic to an oligotrophic state which promoted a higher activity of more well-adapted and competitive bacteria.

4.2 | Significant reductions in fungal: bacterial ratio in soils from infected seedlings

Soils associated with inoculated seedlings had a significantly lower fungal: bacterial biomass ratio, which may be driven by their increased bacterial diversity and biomass. As discussed prior, the shifts in soil bacterial community composition in inoculated seedlings may suggest that select bacterial taxa have a competitive advantage in the soil environment following seedling death. During tree decay, soil bacteria are often the initial colonizers of dead plant tissues due to their ability to utilize readily available sugars (Greaves, 1971). Furthermore, the changes which occur to fungal communities following plant decay are linked to their ability to compete and utilize available resources (Prewitt et al., 2014). Therefore, the higher relative abundance of soil bacteria in inoculated seedlings may indicate that they are better adapted than soil fungi at utilizing the available substrates. Significant declines in soil fungal: bacterial biomass ratio were also observed by Yao et al. (2014) in *E. coli* infested soils. This was attributed to the decline of Actinomycetes, which are known to support populations of soil fungi through their functional roles in C turnover and availability (Ma et al., 2013). Therefore, the significant decreases in the soil fungal: bacterial ratio of inoculated kauri seedlings may be driven in part by their reduced biomass of the Actinomycetes.

One other factor which may have contributed to the declines in soil fungal biomass in inoculated seedlings is the introduction of *P. agathidicida* into the soil environment. *Phytophthora* pathogens are hemibiotrophic pathogens, meaning they have an initial stage of parasitizing living host cells before continuing to live off dead host tissue as saprophytes (Horbach et al., 2011; Thines, 2013). A previous study by Bellgard et al. (2016) observed an increased abundance of *P. agathidicida* within and outside the root zone of infected kauri. These findings are supported by this present study, as all cDNA extracts of soils collected from the surrounding root zone of inoculated seedlings tested positive for *P. agathidicida*. The incorporation of *P. agathidicida* in the soils of inoculated seedlings may have competitively excluded resident fungal communities for space and nutrients at the root zone, thus contributing to the decline in fungal biomass. Root exudates released by plants allow plant rhizospheres to support the activities of soil microorganisms (Baldrian, 2016), and however, when soil pathogens invade new environments, they compete with resident microorganisms for space and nutrients (van Elsas et al., 2012). Compared to the symptomatic kauri studied by Byers, Condron, O'Callaghan, et al. (2020), the smaller root area of kauri seedlings may have increased rates of resource competition and limited the activities of the resident soil fungal communities. As a control variable, this study inoculated 'non-inoculated' kauri seedlings

with blank V8 agar plugs. However, future studies may wish to inoculate control seedlings with a non-pathogenic *Phytophthora* species so that both inoculated and control seedlings were subject to the same increased rates of resource competition. This form of control could not be used in this study as our current understanding on the range of *Phytophthora* species that are pathogenic against kauri is inconclusive. Despite *P. agathidicida* being identified as the primary causal agent of kauri dieback, several other *Phytophthora* species have also been linked to kauri ill health (Horner & Hough, 2014; Waipara et al., 2013).

4.3 | The taxonomic responses of microbial communities to seedling infection

There were only small differences in the composition of soil fungal communities between inoculated and non-inoculated seedlings. Moreover, the previously reported literature on the disease suppressive properties of the fungal taxa found significantly higher in soils from inoculated seedlings (e.g. *Hyaloscyphaceae* and *Melinomyces*) is limited.

However, several bacterial taxa belonging to the Firmicutes (e.g., *Clostridium*, *Bacillus* and *Paenibacillus*) and the Burkholderiales were found in significantly higher abundance in soils from inoculated seedlings. Members of the Firmicutes have been previously found common to disease suppressive soils (Chapelle et al., 2016; Xiong et al., 2017) and strains of *Bacillus* and *Paenibacillus* have been developed for use as biocontrol agents (Govindasamy et al., 2010; Kim et al., 2009; Ryu et al., 2003). For example, *Bacillus* has been identified to produce anti-fungal compounds which are suppressive against *Phytophthora nicotianae*, such as bacillomycin and surfactin (Ros et al., 2017). Furthermore, several studies have demonstrated *Paenibacillus* and *Bacillus* to antagonize a variety of *Phytophthora* species, such as *P. capsici* (Akgül & Mirik, 2008; Jung et al., 2004), *P. cactorum* (Bae et al., 2004; Utkhede, 1984), *P. palmivora* (Timmusk et al., 2009), *P. nicotianae* (Ren et al., 2012; Wu et al., 2018) and *P. infestans* (Caulier et al., 2018). Several *Burkholderia* species have been identified to produce antibiotics and volatile organic compounds (VOCs) which can antagonize plant pathogens (Schmidt et al., 2009; Tenorio-Salgado et al., 2013). Benítez and Gardener (2009) isolated two novel members of the Burkholderiales which significantly inhibited pathogen growth and suppressed plant lesion development against a range of plant pathogens, including *Phytopthora sojae*. Furthermore, *B. cepacia* has been identified as an effective biocontrol agent against *P. capsici*, the causal agent of late blight in pepper (Sopheareth et al., 2013).

Disease suppression can be induced into soils following disease outbreak due to the selective enrichment of soil microorganisms which possess functional traits suppressive against pathogens (Baker & Cook, 1974; Chapelle et al., 2016; Raaijmakers & Mazzola, 2016; Weller et al., 2002). The suppressive soil memory which can develop after disease outbreak has been used to develop synthetic soil bacterial communities shown to significantly reduce plant disease expression (Bakker et al., 2018; Berendsen et al., 2018). For example, Berendsen et al. (2018) identified that downy mildew infection of *Arabidopsis thaliana* promoted the abundance of the bacterial genera *Microbacterium*, *Stenotrophomonas* and *Xanthomonas*. Co-inoculations of these bacterial taxa were able to induce plant systemic resistance against downy mildew upon further infection. Such findings by Berendsen et al. (2018) offer promising insight into how disease suppression can be induced into soils by enriching the population levels of microbial taxa which exhibit strong responses to plant infection. To extend the findings of this kauri seedling study, the bacterial taxa that showed a strong response to seedling infection could be amended in soils to a higher population density. Kauri seedlings grown in these amended soils could then be infected with *P. agathidicida* to identify if the increased abundance of the bacterial taxa prevented or reduced plant disease expression.

4.4 | Contrasts with previous kauri dieback research

This kauri seedling infection study found pronounced responses from soil bacterial communities versus soil fungal communities to the inoculation of kauri seedlings with *P. agathidicida*. In contrast, Byers, Condron, O'Callaghan, et al. (2020) identified that symptomatic kauri had a significantly higher soil fungal diversity compared to asymptomatic kauri, alongside more pronounced shifts in soil fungal community composition between asymptomatic and symptomatic kauri. The differences in these findings may be a result of the major differences in the environmental characteristics of the two plant systems under study. The experimental conditions of this kauri seedling infection study meant that the soil microbial communities were studied using a closed container pot trial in association with younger, smaller seedlings. When studying the role of soil microorganisms in responding to disease outbreaks, it is important to acknowledge how they function within their ecosystems (Baldrian, 2016). For ecologically unique habitats such as kauri forests, properties of the soil microbiota are shaped by the influences that the dominant tree species exert on their surrounding soil environment (Wyse et al., 2014). The conditions imposed during the seedling experiment may not have elicited the same functional response of the soil fungal communities to host infection as would have occurred when within the influence of their original kauri host. The increased fungal diversity observed in symptomatic kauri soils by Byers, Condron, O'Callaghan, et al. (2020) was attributed to increased outputs of dead plant tissue on the forest floor following tree dieback. However, for this seeding experiment, disease expression did not produce such large outputs of dead plant tissue due to the smaller root, shoot and leaf area of each seedling. Plant litter contributes to the majority of organic matter inputs in forest soils and can support proliferous populations of saprophytic fungi (Baldrian, 2016). The comparatively limited plant litter outputs following seedling mortality could not support such an increase in secondary saprotrophic fungi as observed by Byers, Condron, O'Callaghan, et al. (2020). In addition, influxes of soil

fungal populations following seedling disease expression were restricted as the seedling experiment was a closed container, pot trial which allowed for no large fluctuations of fungal populations. Future studies may wish to perform open container pot trials which allow for greater fluctuations in soil fungal populations in response to disturbance events such as pathogen invasion.

The relatively short 6-week inoculation period that was selected before characterizing the response of the soil microbiota to seedling infection may have contributed to the limited response of soil fungal communities. As discussed prior, soil bacteria are often the initial colonizers of dead plant tissue and it is not until the later stages of plant decay that populations of soil fungi rise as they are better able to utilize partially degraded plant tissues (Prewitt et al., 2014). To build upon the findings of this seedling infection study, future research may aim to study the response of the soil microbiota over longer time periods (i.e., 3 to 6 months) and at multiple time points throughout the inoculation period. This would allow us to characterize the successional changes of soil microbial communities over time, which may provide more information about the response of soil fungal communities to kauri infection.

5 | CONCLUSION

This study aimed to observe the short-term responses of the soil microbiota 6 weeks following the infection of kauri seedlings with *P. agathidicida*. Seedlings inoculated with *P. agathidicida* experienced pronounced and rapid shifts in soil bacterial community composition. The bacterial taxa found in significantly higher relative abundance in soils from inoculated seedlings, which includes several members of the Firmicutes, have previously reported roles in plant disease suppression. Before we can begin assessing the potential of these taxa to function as biocontrol agents against *P. agathidicida*, their interaction with *P. agathidicida* needs to be studied in a more targeted manner. Soil fungal communities associated with kauri seedlings inoculated with *P. agathidicida* showed only minor responses to seedling infection. This contrasts to previous field-based research which observed a strong response of soil fungal communities to the expression of kauri dieback in mature kauri. The results of this kauri seedling infection study identified members of the soil microbiota that were the primary respondents to plant infection. However, to better understand the changes in microbial community dynamics following seedling infection, particularly for soil fungal communities, future studies should study the response of the soil microbiota over a longer time period.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. Any further data of interest that supports the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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